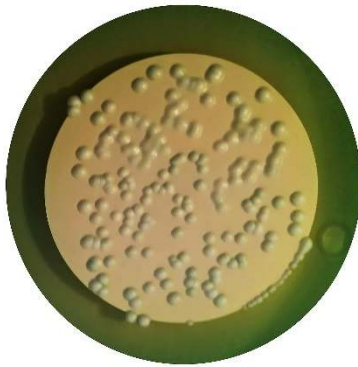




m-GREEN AGAR

Dehydrated and ready-to-use culture medium



m-Green Agar: membrane filter
with colonies of *Saccharomyces cerevisiae*

1 - INTENDED USE

For the enumeration of yeasts and moulds in beverages, with membrane filter method.

2 - COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Yeast extract	9.0 g
Dextrose	50.00 g
Peptone	10.00 g
Magnesium sulphate	2.10 g
Potassium phosphate	2.0 g
Diastase	0.05 g
Thiamine hydrochloride	0.05 g
Bromocresol green	0.026 g
Agar	23.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

m-Green Agar is a complex composition medium used by the beverages industry for the detection and enumeration of yeast and moulds by the membrane filter method. It is prepared by adding agar to the formulation reported by ISO 10718¹ for counting yeasts and moulds, capable of both being extracted and growing in alcoholic medium, in cork stoppers.

Peptone and yeast extract provide the nitrogen, and amino acids, vitamins and minerals for microbial growth. Dextrose is a source of carbon and energy. Potassium phosphate buffers the medium. Magnesium sulphate, thiamine, and diastase (a mixture containing amylolytic enzymes) provide important ions, minerals, and nutrients for mycological growth. The composition includes bromocresol green which facilitates the visualization and counting of fungal colonies that are green due to diffusion of the stain into the colonies. Acidic end products from colonies diffuse into the medium, reducing the pH and causing the indicator to turn yellow (acid reaction) around the colonies. The growth of non-acidophilic bacteria is inhibited by the acidic pH of the medium.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 96.7 g in 1000 mL of cold purified water; heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Mix well and pour into sterile Petri dishes. Prolonged or excessive heating will diminish the gel strength of the agar.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	greenish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	green, clear
Final pH at 20-25 °C	4.6 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
m-Green Agar	Dehydrated medium	4015272	500 g (5.1 L)
m-Green Agar	Ready-to-use plates	491527	3 x 10 plates Ø 55 mm

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, membrane filtration system, ancillary culture media and reagents.

8 - SPECIMENS

Non-alcoholic and alcoholic beverages. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

Filter an appropriate volume of sample onto the membrane, depending on the expected yeast and mould charge.
Roll the membrane onto the surface of the agar, so as to avoid the formation of air bubbles between the filter and the agar surface.
Incubate at 30 °C ± 2 °C for 72 hours.

10 - READING AND INTERPRETATION

Enumerate the number of colonies of yeasts and moulds per plate and calculate the microbial count.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T° / T - ATM	EXPECTED RESULTS
<i>S. cerevisiae</i> ATCC 9763	28-32° / 72 H-A	good growth, pale green colonies
<i>A. brasiliensis</i> ATCC 16404	28-32° / 72 H-A	good growth
<i>B. subtilis</i> ATCC 6633	28-32° / 72 H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection





12 – PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated and ready to use m-Green Agar (Test Batch:TB) are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch and Sabouraud Dextrose Agar (SDA). Productivity is assessed by a quantitative test with the following strains: *S. cerevisiae* ATCC 9763, *C. albicans* ATCC 10231, *C. tropicalis* NCPF 8841, *A. brasiliensis* ATCC 16404. The filters on the plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 28-32°C for 72 hours. The colonies are enumerated on both batches and the productivity ratio (Pr:CFU_{TB}/CFU_{SDA}) is calculated. If Pr is ≥ 0.5 and if the colonies morphology and colour are typical the results are considered acceptable and conform to the specifications. The selectivity is assessed with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of *B. subtilis* ATCC 6633. The growth of the non-target strain is inhibited

13 – LIMITATIONS OF THE METHOD

- Avoid over-heating and remelting of medium.
- The isolated colonies on the plates should be identified with suitable tests.

14 - PRECAUTIONS AND WARNINGS

- This culture medium is for Laboratory use and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- Each ready-to-use plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheets of the products are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Ready to use plates

Upon receipt, store plates in their original pack at +2°C /+8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

Dehydrated medium

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type and the applied storage conditions (temperature and packaging).

16 – REFERENCES

1. ISO 10718:2015 Cork stoppers – Characterization of a low-in-germs stopper, through the enumeration of colony-forming units of yeast, moulds and bacteria, capable of both being extracted and growing in alcoholic medium.

TABLE OF APPLICABLE SYMBOLS

or Catalogue number	Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/08

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

